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54. Title of the invention:

Method of producing protein having factor VII activity

57. Abstract:

Problem: Novel method of producing protein having factor VIIa activity.

Solution: Method characterized in that mammalian cells are cultured into which DNA coding for protein having factor VIIa activity after activation has been inserted, and protein is obtained from this culture fluid and activated.

#### PATENT CLAIMS:

1. A method of producing protein having biological activity for blood coagulation mediated by factor VIIa comprising growing in an appropriate culture medium mammalian host cells containing a DNA construct containing a nucleotide sequence coding for a protein having the same or essentially the same biological activity for blood coagulation as factor VIIa having the following amino acid sequence:

[see extra sheet]

isolating the protein product encoded by said DNA construct and produced by said mammalian host cells, and activating said protein product and generating protein which has the same or substantially the same biological activity for blood coagulation as factor VIIa.

2. A method according to claim 1, including amplification of said DNA construct by cotransfection of said host cells with a gene coding for dihydrofolate reductase, wherein said appropriate medium contains methotrexate.

3. A method according to claim 1, wherein said protein product is activated by reacting it with a proteolytic enzyme selected from the group consisting of factor XIIa, factor IXa, kallikrein, factor Xa, and thrombin.

|             |                         |                 |         |
|-------------|-------------------------|-----------------|---------|
| 1           | 5                       | 10              | 15      |
| Glu Cys Lys | Glu Glu Gln Cys Ser Phe | Glu Glu Ala Arg | Glu Ile |
| 20          | 25                      | 30              |         |
| Phe Lys Asp | Ala Glu Arg Thr Lys Leu | Phe Trp Ile Ser | Tyr Ser |
| 35          | 40                      | 45              |         |
| Asp Gly Asp | Gln Cys Ala Ser Ser Pro | Cys Gln Asn Gly | Gly Ser |
| 50          | 55                      | 60              |         |
| Cys Lys Asp | Gln Leu Gln Ser Tyr Ile | Cys Phe Cys Leu | Pro Ala |
| 65          | 70                      | 75              |         |
| Phe Glu Gly | Arg Asn Cys Glu Thr His | Lys Asp Asp Gln | Leu Ile |
| 80          | 85                      | 90              |         |
| Cys Val Asn | Glu Asn Gly Gly Cys Glu | Gln Tyr Cys Ser | Asp His |
| 95          | 100                     | 105             |         |
| Thr Gly Thr | Lys Arg Ser Cys Arg Cys | His Glu Gly Tyr | Ser Leu |
| 110         | 115                     | 120             |         |
| Leu Ala Asp | Gly Val Ser Cys Thr Pro | Thr Val Glu Tyr | Pro Cys |
| 125         | 130                     | 135             |         |
| Gly Lys Ile | Pro Ile Leu Glu Lys Arg | Asn Ala Ser Lys | Pro Gln |
| 140         | 145                     | 150             |         |
| Gly Arg Ile | Val Gly Gly Lys Val Cys | Pro Lys Gly Glu | Cys Pro |
| 155         | 160                     | 165             |         |
| Trp Gln Val | Leu Leu Val Asn Gly     | Ala Gln Leu Cys | Gly Gly |
| 170         | 175                     | 180             |         |
| Thr Leu Ile | Asn Thr Ile Trp Val Val | Ser Ala Ala His | Cys Phe |
| 185         | 190                     | 195             |         |
| Asp Lys Ile | Lys Asn Trp Arg Asn Leu | Ile Ala Val Leu | Gly Glu |
| 200         | 205                     | 210             |         |
| His Asp Leu | Ser Glu His Asp Gly Asp | Glu Gln Ser Arg | Arg Val |
| 215         | 220                     | 225             |         |
| Ala Gln Val | Ile Ile Pro Ser Thr Tyr | Val Pro Gly Thr | Thr Asn |
| 230         | 235                     | 240             |         |
| His Asp Ile | Ala Leu Leu Arg Leu His | Gln Pro Val Val | Leu Thr |
| 245         | 250                     | 255             |         |
| Asp His Val | Val Pro Leu Cys Leu Pro | Glu Arg Thr Phe | Ser Glu |
| 260         | 265                     | 270             |         |
| Arg Thr Leu | Ala Phe Val Arg Phe Ser | Leu Val Ser Gly | Trp Gly |
| 275         | 280                     | 285             |         |
| Gln Leu Leu | Asp Arg Gly Ala Thr Ala | Leu Glu Leu Met | Val Leu |
| 290         | 295                     | 300             |         |
| Asn Val Pro | Arg Leu Met Thr Gln Asp | Cys Leu Gln Gln | Ser Arg |
| 305         | 310                     | 315             |         |
| Lys Val Gly | Asp Ser Pro Asn Ile Thr | Glu Tyr Met Phe | Cys Ala |
| 320         | 325                     | 330             |         |
| Gly Tyr Ser | Asp Gly Ser Lys Asp Ser | Cys Lys Gly Asp | Ser Gly |
| 335         | 340                     | 345             |         |
| Gly Pro His | Ala Thr His Tyr Arg Gly | Thr Trp Tyr Leu | Thr Gly |
| 350         | 355                     | 360             |         |
| Ile Val Ser | Trp Gly Gln Gly Cys Ala | Thr Val Gly His | Phe Gly |
| 365         | 370                     | 375             |         |
| Val Tyr Thr | Arg Val Ser Gln Tyr Ile | Glu Trp Leu Gln | Lys Leu |
| 380         | 385                     | 390             |         |
| Met Arg Ser | Glu Pro Arg Pro Gly Val | Leu Leu Arg Ala | Pro Phe |
| 395         | 400                     | 405             |         |

Pro

Figure 5 illustrates the Factor VII cDNA sequence of  $\lambda$ VII 2463.

Figure 6 illustrates the Factor VII cDNA sequence of  $\lambda$ VII 2463.

Figure 7 illustrates the Factor VII cDNA sequence of  $\lambda$ VII 2463.